

Human TLR9 Agonist Kit

Set of known agonists for human TLR9

Catalog # tlr1-kit9h

For research use only

Version # 16F09-MM

PRODUCT INFORMATION

Content:

ODNs are provided lyophilized:

- 100 µg (13.0 nmol) ODN 2006 (ODN 7909)
- 100 µg (13.0 nmol) ODN 2006 Control (ODN 2137)
- 100 µg (15.5 nmol) ODN 2216
- 100 µg (15.5 nmol) ODN 2243 (ODN 2216 Control)
- 100 µg (14.2 nmol) ODN 2395
- 100 µg (14.2 nmol) ODN 2395 Control
- 1.5 ml endotoxin-free water

Storage and stability:

- Products are shipped at room temperature and should be stored at -20°C.
- Upon resuspension, prepare aliquots of ODN and store at -20°C. Resuspended product is stable for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

- TLR9 activity has been tested using HEK-Blue™ TLR9 cells.
- The absence of bacterial contamination (e.g. lipoproteins & endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

CpG ODNs are synthetic oligonucleotides that contain unmethylated CpG dinucleotides in particular sequence contexts (CpG motifs). These CpG motifs are present at a 20-fold greater frequency in bacterial DNA compared to mammalian DNA¹. CpG ODNs are recognized by Toll-like receptor 9 (TLR9) leading to strong immunostimulatory effects.

- **ODN2006 (also known as ODN 7909 or PF-3512676)** is a class B CpG ODN with a preference towards human TLR9. Class B CpG ODNs contain a full phosphorothioate backbone with one or more CpG dinucleotides. They strongly activate B cells but weakly stimulate IFN-α secretion.
- **ODN 2006 Control (also known as ODN 2173)** contains GpC dinucleotides instead of CpGs and can be used as a negative control together with ODN 2006. *Note: In some cell types, ODN 2006 Control may stimulate cell activity, including the production of cytokines².*
- **ODN 2216** is a CpG ODN class A with a preference towards human TLR9. Class A CpG ODNs are characterized by a phosphodiester central CpG-containing palindromic motif and a phosphorothioate 3' poly-G string. They induce high IFN-α production from plasmacytoid dendritic cells (pDC) but are weak stimulators of TLR9-dependent NF-κB signaling.
- **ODN 2243 (also known as ODN 2216 Control)** contains GpC dinucleotides instead of CpGs and can be used as a negative control together with ODN 2216.
- **ODN 2395** is a CpG ODN class C for human and mouse TLR9. Class C CpG ODNs combine features of both classes A and B. They contain a complete phosphorothioate backbone and a CpG-containing palindromic motif. Class C CpG ODNs induce strong IFN-α production from pDC and B cell stimulation.
- **ODN 2395 Control** contains GpC dinucleotides instead of CpGs and can be used as a negative control with ODN 2395.

1. Bauer, S. *et al.*, 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. PNAS 98(16):9237-42. 2. Reid G. *et al.*, 2005. CpG stimulation of precursor B-lineage acute lymphoblastic leukemia induces a distinct change in costimulatory molecule expression and shifts allogeneic T cells toward a Th1 response. Blood 105(9):3641-7.

TECHNICAL SUPPORT

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SEQUENCES

ODN 2006 (class B): 5'- tcgtcgttttgcgttttgcgtt-3' (24 mer)

ODN 2006 Control: 5'- tgctgcttttgcgttttgcgtt-3' (24 mer)

ODN 2216 (class A): 5'- ggGGGACGA:TCGTCgggggg-3' (20 mer)

ODN 2243 (ODN 2216 Control): 5'- ggGGGAGCATGCTGgggggg-3' (20 mer)

ODN2395 (class C): 5'- tcgtcgttttggcgc:gcgccg-3' (22 mer)

ODN 2395 Control: 5'- tgctgcttttggggggccccc -3' (22 mer)

Note: Bases shown in capital letters are phosphodiester, those in lower case are phosphorothioate (nuclease resistant) and palindrome is underlined.

METHODS

Preparation of stock solution (500 µM)

Product	Molecular weight	Working conc	Stock solution	Volume of solvent
ODN 2006 (ODN 7909)	7698 g/mol	5 µM	500 µM	26 µl H2O
ODN 2006 Control	7698 g/mol	5 µM	500 µM	26 µl H2O
ODN 2216	6393 g/mol	5 µM	500 µM	31 µl H2O
ODN 2243 (ODN 2216 Control)	6472 g/mol	5 µM	500 µM	31 µl H2O
ODN 2395	7048 g/mol	5 µM	500 µM	28 µl H2O
ODN 2395 Control	7048 g/mol	5 µM	500 µM	28 µl H2O

- Resuspend ODN with endotoxin-free water provided.

CpG ODN stimulation

ODNs can be used to stimulate TLR9 in HEK-Blue™ TLR9 cells. HEK-Blue™ TLR9 cells stably overexpress the TLR9 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP).

For more information, visit: www.invivogen.com

Below is a protocol to study TLR9 stimulation using HEK-Blue™ TLR9 cells in a 96-well plate.

- Dispense 20 µl of stimulatory or control ODN per well of a 96-well plate.
- Prepare HEK-Blue™ TLR9 cell suspension according to the data sheet.
- Add HEK-Blue™ TLR9 cells (4-8 x10⁴) to each ODN-containing well.
- Incubate for 6-24 h at 37 °C, 5% CO₂.
- Determine TLR9 stimulation by assessing cytokine expression using ELISA, or SEAP expression using QUANTI-Blue™, a SEAP detection medium.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR9 cells	hkb-hltr9
ODN 2006	tlr1-2006
ODN 2216	tlr1-2216
ODN 2395	tlr1-2395